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14. ABSTRACT
At present, there are substantial concerns about the impacts of anthropogenic sound in the oceans, and there is a particular focus by the public on impacts from military sonars which is having a substantial practical and fiscal impact on US Navy operations. Multiple research approaches are being employed to address the question of the possible extent and mechanisms for acoustic impacts, but progress has been slow. In part this is due to the multiplicity of organisms that may be affected and the difficulty in making repeat, controlled measures of impacts in the majority of sea creatures under real world conditions.

15. SUBJECT TERMS
Marine mammal, cetaceans, biomedical imaging, databases, sonar, FEM, noise impacts, strandings, head and neck trauma, pathology, barotraumas, CT, MRI, pinnipeds, acoustic resonances, target strengths, veterinary medicine, dive physiology, stress lesions

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Database Development for Ocean Impacts: Imaging, Outreach, and Rapid Response

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LONG-TERM GOALS

The overall goal for this project is to provide a web-accessible database on the anatomy and physiology of marine mammals, their prey species, and other endangered marine species for which there are concerns for underwater acoustic impacts.

OBJECTIVES

A principal objective of this project is to provide a resource for the research community to improve accuracy of models and representations of marine mammal anatomy and standardization of measures taken during strandings which will as improve our broader understanding and response efficacy for stranding events. This multi-year effort will provide to the scientific community, stranding network, and public a research resource, educational tutorials at the lay and professional research level, professional clinical case histories, and interpretative manuals on imaging procedures, related research, and determination of normal vs abnormal findings for *in vivo* and post mortem exams of marine mammals and sea turtles.

WORK COMPLETED

The most recent version of the database which was supported through this funding (http://csi.whoi.edu) was launched publicly May 2011. It is hosted on a local WHOI Apache web server (cetus.whoi.edu) configured to host the Drupal based Content Management System (CMS)/Image Database for the long term. Site traffic is monitored daily by Google Analytics. From May to August 31, 2011 the site produced over 1900 total visits (1400 unique visits; 72 % new visitors, 28 % returning visitors), 27,000 page views and over 160 downloads of images and content worldwide. XML site maps have been configured to automatically update several of the major search engines including Google, Yahoo, and Bing. This design will improve internet visibility with eacb search engine indexing session.
Base configuration of a dynamic MySQL database structure that serves the website content is complete with security and backup configurations updated daily by WHOI Computer Information Services.

The site currently bolds over 3500 images, 43 animations/movies and 116 publications available for review and download. Content included in the database and available to end users includes CT images and digital photos of the scanning process and field and laboratory dissections of selected cases as well as video galleries of 3D reconstructions and animations obtained from CT scans. These images and case material are linked to audio galleries of sound recordings of some marine mammal species and for specimens for which necropsy reports and data are available with their case files for registered users. Records of CT service requests, specimen inventories, and image archive inventories are also available on the site. .

The image sets cover 170 species of marine and land mammals, including approximately 400 data sets for cetaceans alone. Making this database available to the scientific community is critical for several research fronts attempting to solve acoustic impact issues; e.g., finite element and finite difference models (FEM/FDM) of acoustic propagation characteristics of different species, modeling tissue responses to over exposure, understanding differences amongst species for specific sound sources, and the education of stranding responders

and pathologists in the recognition and interpretation of normal variants vs. pathologies in different post-mortem stages for marine mammals.

Current features of the new image database website include but are not limited to:

- User Login and Registration - (allows website administrators to control access to confidential case data.)
- User Profiles
- Full Boolean search capabilities for all content including images, multimedia and text files.
- Literature and specimen case files with multiple search options; e.g., sort by publication type, year, author, title, keyword, etc.; export/import of citations in multiple formats; abstract and full text viewing capabilities; download full text or abstract as PDF files and upload of citations by users in multiple formats including EndNote, Tagged, XML and BibTex.
- Image and video galleries with sample images and videos obtained from CT scans, 3D reconstructions and field and laboratory dissections as well as links to specific case images and data for individual specimens.
- Audio galleries with sample recordings of multiple species.
- Embedded PDF documents viewable without third party software
- Imaging request forms for all CT service requests by outside users
- Web form reports for administrators and laboratory staff displaying website CT service requests as spreadsheets and tables in multiple formats including xcl, dba, acc and mysql.
- RSS feed aggregators for WHOI News and BBC News on marine species and events
- Categorized forums for interdisciplinary discussions.
- Content commenting for website user feedback and questions.
- Event Calendars - provide users with a list of upcoming interdisciplinary events for groups both within and outside WHOI. It also integrates iCal for use with thunderbird, outlook, apple mail, iPod, and entourage.
- Multiple embedded flash components for viewing web pages and search pages of other sites through this site.
- Categorized FAQ (frequently asked questions) section
- Create Content -This option gives website administrators the ability to allow content creation by website users such as for events, publications, comments, forums, images and videos based on user profile criteria
- Integrated links to WHOI and other related websites and funding organizations

Content for the site is updated daily and new content is added weekly as additional resources become available. Suggestions from end users are welcome and have been helpful for improving website performance and usability. Further website developments anticipated include interactive 3D animations and annotated anatomy with on-line tests available for students.

Database Development

The database at present provides preliminary image sets and annotations at the lay level for a limited range of taxa. More advanced, research and treatment related cases are available under the sections on pathologies as well as data sets and images on FEM model development in the video, 3D, and model sections of the website. These images are linked to extensive case file data that provide more advanced research relevant content and are accessible primarily to registered users.

Datasets Added / Available via the Website

A total of 866 specimens were scanned under the funding of this project, with 274 scanned in the last year alone. Of these, 52 cases were live or post mortem strandings. The cases imaged to date included the following:

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- Coral cores for climate change research (A. Cohen, WHOI, PI and visiting faculty and students from The Australian Nuclear Science & Technology Organization, The Biology Department at University of Puerto Rico & The Department of Earth and Planetary Sciences at University of California, Santa Cruz).
- Stretch hoses (Applied Ocean Physics & Engineering department, WHOI, to evaluate wear and locate in mooring optical cables used in the Right Whale monitoring program)
- Stromatolites for climate change research (J. Bernard, A. McIntyre-Wressnig, WHOI, PI)
- Humboldt squid (*Dosodicus gigas*)(Iliana Ruiz-Cooley,, NOAA-Southwest Fisheries Science Center to determine sensory system anatomy)
- Live Midshipman and Lusitanian toadfish (R. Fay and R. Sisneros; Marine Biology Laboratory visiting post-doctoral and Grass Fellows, to evaluate otolith structure and relationships to the swimbladder).
- Oil samples from the Deepwater Horizon spill (C Reddy , Marine Chemistry & Geochemistry Department ,WHOI, to evaluate pattem of rippling and visualize internal structure of deposits washing ashore)
- Chondricthyes (Rays and Shark) for fin design and hydrodynamics (F.Fish in association with Robert Russo, University of Virginia Department of Mechanical & Aerospace Engineering, PI)
- Narwhal flippers and flukes (F. Fish in association with Natalia Rybczynski, Canadian Museum of Nature-Research/ Paleobiology)
- Seals and pigs in pressure chamber (A. Fahlman, M. Moore, WHOI, PI)
- Odontocetes and seals for IFAW/NOAA NMFS for stranding diagnostics
- Turtle ear 3D reconstructions and fat volumes for hearing study (C. Carr, U. MD, PI)
- Terrapin diagnoses, *in vivo,* for National Marine Life Center (S. Rogers Williams, DVM)
- Blue whale ears for stranding evaluation (J. Jacobsen, Humboldt State)
- Shipworm infestation experiments (S. Gallagher, WHOI, PI)
- Minke whale head tissues (D.Ketten, WHOI, PI) o
- *Ziphius cavirostris* ears, stranding evaluations (N. Hauser, Cook Islands, PI)
- Tiger ears for LF hearing studies (E. Walsh, Boystown, PI)
- Neophocoena phocaenoides stranding evaluations (W. Ding and D. Ketten, China, PI)
- Sand lances for summer student project on cetacean prey species (S. Strobel, WHOI)
- Cetacean and Chiropteran micro CT scans of the inner ear (for ultrasonic adaptatipons, in collaboration with J Simmons, Brown Univ.)
- Rubber stretch hose to evaluate defects in deep water buoys (L. l O'Hara, D. Peters, WHOI, PI)
- Micro-circuit boards to evaluate crystal defects (E. Gallimore, WHOI, PI)
- Tissue segmentations of whole cetaceans (K. Foote, WHOI, PI)

RESULTS

Unlike most research contracts and proposals, the principal output of this project is not a set of incremental discoveries but rather to categorize and annotate existing data sets acquired for individual projects and as part of diagnostic procedures for stranded animals. The first year of this project was devoted largely to completion of the design and to testing accuracy and efficiency of each component in an operational website. The majority of the second and third years were devoted to transitioning existing data to a web-accessible database and increasing the sophistication of the website as well as testing security of proprietary data for individual researchers. In this process and to increase the value of the data, manuals on the scanning procedures used and interpretations of the data sets were completed and published. The last year was focused on the transitioning of

major filesets for a full range of species and exploration of new protocols for challenging imaging subjects; e.g., high density, multi-meter coral cores, oarfish, live invertebrates, deep sea cabling, and deep sea cores.

IMPACT/APPLICATIONS

The potential for scan data is illustrated by the examples below taken from recent studies within this laboratory. As indicated by the datasets listed above an by the publications list below, scan data is assisting a wide range of ONR researchers and ranges from climate change studies to in vivo diagnostics for determination of rehabilitation potentials and outcomes.

Macro to Micro: Whole Body to Inner Ear

In Figure 1, 3D reconstructions of two species of odontocetes demonstrate significant differences in the structure, volume, and content of tissues that are critical to sound reception. By segmenting tissues in the heads of each species, based on their X-ray attenuations, which correspond to tissue sound transmission, it is possibe to determine geometries fundamental to the frequency and acoustic attenuation characteristics. In both cases, fatty tissues are found to be pinnal analogues but also that these fatty "pinnae" are species specific. This implies that, like the pinnae and outer ear canals of land mammals, the fatty tissues are critical determinants of peak resonances and thus sensitivities of each species.

Figure 1. In each figure, a reconstruction of scans of the head of an intact specimen reveals the skull anatomy (transparent white), two components of the melon (outer layer green and inner core purple), and the multi-lobed fats aligning with and surrounding the mandible (gold) (see also: Ketten, D.R. 2008 Underwater ears and the physiology of impacts: Comparative liability for hearing loss in sea turtles, birds, and mammals. Bioacoustics, vol. 17, no. 1-3, pp. 312-315).

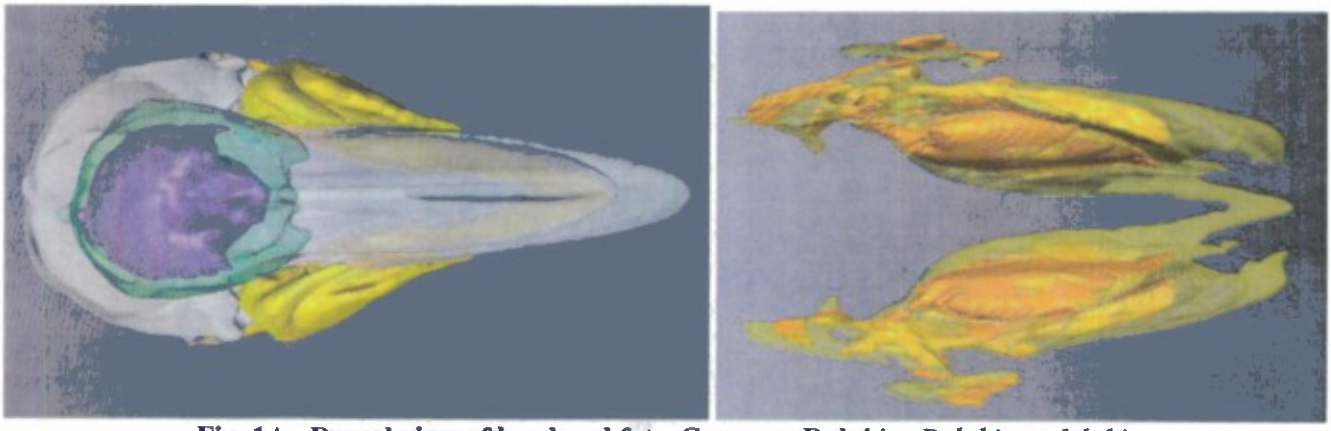


Fig. 1A. Dorsal view of head and fats, Common Dolphin, *Delphinus delphis*

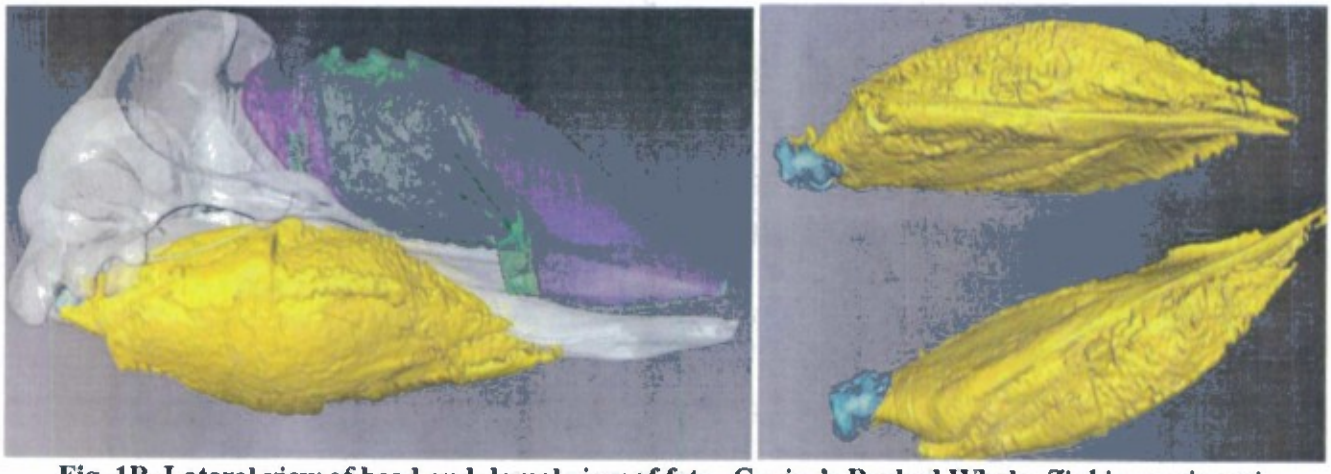


Fig. 1B. Lateral view of head and dorsal view of fats, Cuvier's Beaked Whale, *Ziphius cavirostris*

Hearing Loss in Cetaceans

In Figure 2, CT images and 3D reconstructions of the inner ear of bottle nose dolphins are used to calculate sites of absence of auditory nerve fibers and thus corresponding frequencies of hearing loss in older animals. Comparisons of the predicted loss maps with the hearing curves of these animals show perfect correspondance for the maps with hearing abilities measured behaviourally. This exercise demonstrates the accuracy and potential for CT exams to determine the presence or absence of hearing deficits in stranded animals, pre or post mortem.

Figure 2. *Tursiops truncatus* inner ear imaged with CT to obtain frequency of hearing loss. Figure 2A shows the basilar membrane (green) auditory nerve (orange), and ganglion cells and fiber (purple) distributions. Estimates of frequency calculated for this ear are listed by position on the membrane. The lack of fibers beyond ~57 kHz suggests that this was the high frequency functional cutoff for this animal in its later life. A graph (Fig. 2B) shows the curve calculated for this ear for the frequency distributions. Celloidin histology of the ear confirmed the loss. Comparisons with the actual hearing responses show that this form of diagnosis with CT accurately predicts sensorineural hearing loss from aging and noise in this animal. (see also Ketten, D. R., Arruda, ,J. S. Cramer, Dunn, M., and Ridgway, S. 2010 Mature Mammal Hearing Loss: A Natural Experiment in Presbycusis. Association for Research in Otolaryngology, Anaheim Calif.).

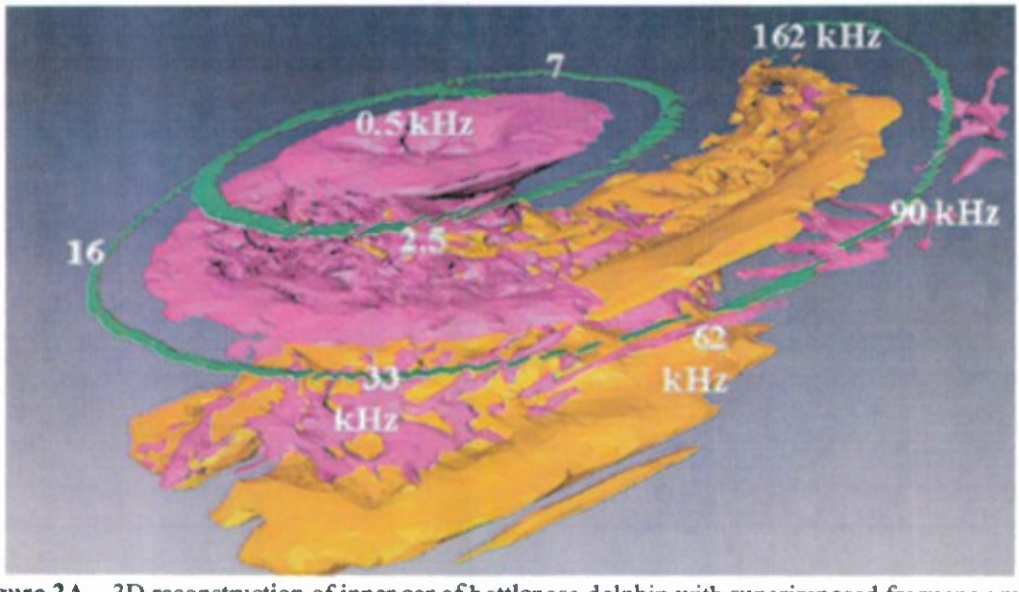
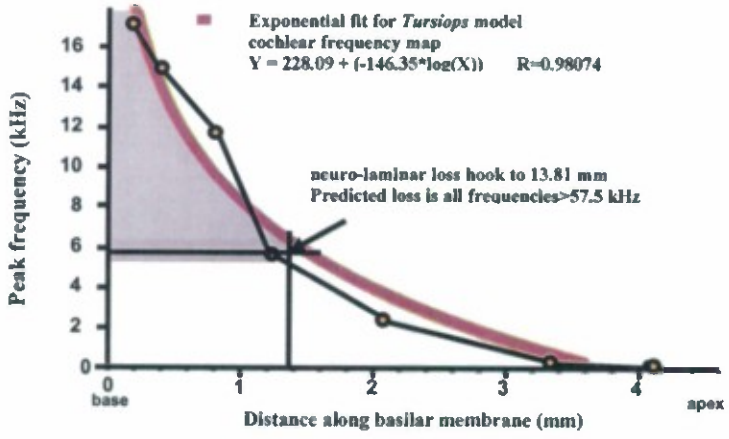


Figure 2A. 3D reconstruction of inner ear of bottlenose dolphin with superimposed frequency map.

Figure 2B. Calculation of bottlenose dolphin frequency map and position of ganglion cell losses related to hearing loss.



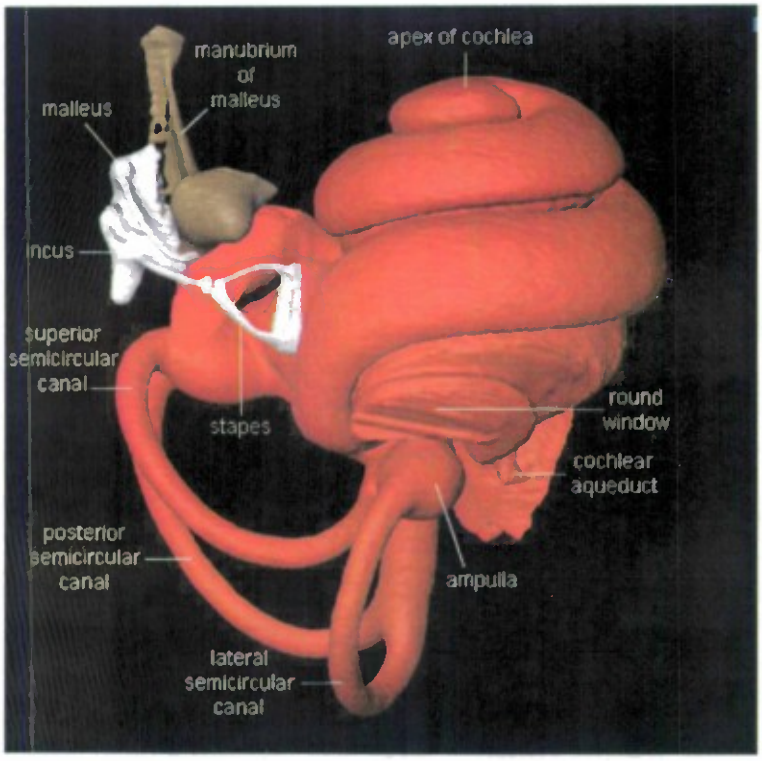


Fig. 3A. A micro CT of the inner ear of a microchiropteran bat, Eptesicus fuscus is shown in a ventral view. The 3D reconstruction was created from 1700, 18 micron thick sections and demonstrates the relationship and relative dimensions of the major inner ear elements as well as the ossicular chain. The cochlea in this specimen is 16 mm in length.

Fig. 3B. The inner ear of the highly endangered Right Whale (*Eubalaena glacialis*) is shown in a 3D view that reveals the cochlea, facial nerve, auditory nerve, and vestibular elements within the periotic bone. The reconstruction was imaged from 800, 100 micron thick sections. The cochlea in this specimen is 56 mm. in length.

RELATED PROJECTS

The methods developed (and personnel employed in the design and implementation) in this database project have been employed to assist in the formation of a second website for the WHOI Marine Mammal Center which will host a website for the dissemination of marine mammal sound data (P. Tyack, PI, N000140710988).

Segmentation data from scan datasets and their analyses for tissue volumes and densities have also been employed in the following ONR projects: lung tissues analyses, Fahlman N000141010791; multi-tissue segmentations, Foote N000140910482; imaging of tissue morphometry under pressure, Moore N000140811220. Other PIs with ONR affiliated projects include hydrodynamics of marine fishes and mammals (F. Fish) and swim bladder morphology (J. Webb) as well as contributing to the projects listed under datasets added, particularly those related to biosonar (Simmons) and fish auditory systems (Fay and Sisneros).

Publications during the last 3 years related to these projects employing website data are listed below.

PUBLICATIONS (downloadable pdfs http://csi.whoi.edu)

Refereed Journals

2009 D'Amico, A. D., Gisiner, R., Ketten, D. R., Hammock, J. A., Johnson, C., Tyack, P. & Mead, J. Beaked whale strandings and naval activities. *Aquatic Mammals*. vol. 35 no. 4 pp. 452-472.

2009 Filadelfo R, Mintz, J., Michlovich E, D'Amico AD, Tyack P, Ketten DR. Correlating military sonar use with beaked whale mass strandings: what do these historical data show *Aquatic Mammals*, . 35 no. 4 pp. 435-444.

2009 Filadelfo, R., Pinelis, Y. K., Davis, S., Chase, R., Mintz, J., Wolfanger, J., Tyack, P. L., Ketten, D. R., and D'Amico, A. Correlating whale strandings with Navy excercises off Southern California. *Aquatic Mammals* vol. 35 no. 4 pp. 452-472.

In Press

2010 Webb, J.F., Shearman, E., Walsh, R.. Ketten, D.R., and Herman, J.L. Inner Ear and Swim Bladder Morphology Associated with a Novel Sensory Specialization in Chaetodontid Fishes. (accepted, *Comp. Integr. Biol.* in press).

2010 Ketten, D.R. Marine Mammal Auditory System Noise Impacts: Evidence and Incidence. In: Proceedings of the Second International Conference on the Effects of Noise on Aquatic Life, A. Popper and T. Hawkins (eds). Springer-Verlag (accepted, in press).

2010 Fontella, J, FE Fish, N Rybczynski, M Nweeia, and DR Ketten,. Three-Dimensional Geometry of the Narwhal (*Monodon monoceros*) Flukes in Relation to Hydrodynamics. (accepted, *Marine Mammal Science*, in press).

2010 Mooney, TA, Hanlon, RT, Christensen-Dalsgaard, J, Madsen, PT, Ketten, DR, Nachtigall, PE (2010). "The potential for sound sensitivity in cephalopods." In: Proceedings of the Second International Conference on the Effects of Noise on Aquatic Life, A. Popper and T. Hawkins (eds). Springer-Verlag (accepted, in press)

2010 Mooney, TA, Hanlon, RT, Christensen-Dalsgaard, J, Madsen, PT, Nachtigall, PE, Ketten, DR. 2010. Sound detection by the longfin squid (*Loligo pealeii*) studied with auditory evoked potentials: sensitivity to low-frequency particle motion and not pressure.(accepted, *Journal of Experimental Biology*, in press.)

Refereed Short Communications

2010 Ketten, D.R. Marine Mammal Auditory System Noise Impacts: Evidence and Incidence. 2nd International Meeting on the Effects of Noise on Aquatic Life. Cork, Ireland.

2010 Mooney, TA, Hanlon, RT, Christensen-Dalsgaard, J, Madsen, PT, Ketten, DR, and Nachtigall, PE. The hearing of the longfin squid (*Loligo pealeii*) and sensitivity to low frequency noise. 2nd International Meeting on the Effects of Noise on Aquatic Life. Cork, Ireland.

2010 Ketten , D.R., J. Arruda, S. Cramer, and M. Dunn Cochlear Morphometrics from CT: Length, Insertion, and Neuronal Distribution Estimates. Objective Measures in Auditory Implants, 6th International Symposium, St. Louis, Mo. USA

2010 Christensen-Dalsgaard, J, CE Carr,, PT Madsen, C Brandt, K Willis, D Ketten, P Edds-Walton, R R. Fay Specialisation for underwater hearing in the red-eared slider turtle, *Trachemys scripta elegans* Association for Research in Otolaryngology, Anaheim Calif.

2010 Christensen-Dalsgaard, J, CE Carr,, PT Madsen, C Brandt, K Willis, D Ketten, P Edds-Walton, R R. Fay Underwater hearing in the red-eared slider turtle, *Trachemys scripta elegans.* International Society for Neuroethology

2010 Ketten, D. R., Arruda, ,J., S. Cramer, Dunn, M., and Ridgway, S. Mature Mammal Hearing Loss: A Natural Experiment in Presbycusis. Association for Research in Otolaryngology, Anaheim Calif.

2010 Mooney, TA, Hanlon, RT, Christensen-Dalsgaard, J, Madsen, PT, Ketten, DR and Nachtigall, PE. What squid hear: An evoked potential study of the longfin squid (Loligo pealeii). Association for Research in Otolaryngology, Anaheim Calif.

2010 Vigness-Raposa, KJ, G Scowcroft, J H Miller, DR Ketten Discovery of Sound in the Sea: An On-line Resource. 2nd International Meeting on The Effects of Noise on Aquatic Life. Cork, Ireland.

2010 Tubelli, A., A. Zosuls, D. R. Ketten and D. C. Mountain. Prediction of a Mysticete Audiogram via Finite Element Analysis of the Middle Ear. 2nd International Conference of the Effects of Noise on Aquatic Life, Cork, Ireland

2010 Zosuls, A., S. O. Newburg, D. R. Ketten and D. C. Mountain. Reverse Engineering the Cetacean Ear to Extract Audiograms. 2nd International Conference of the Effects of Noise on Aquatic Life, Cork, Ireland.

2011 Mooney, TA and DR Ketten Using Auditory Evoked Potentials to Examine Hearing Loss in Aquatic Animals: From Marine Mammals to Squid. Assoc. for Rsch in Otolaryngology, Baltimore, Md.

2011 Fontanella, J. E., Fish, F. E., Rybczynski, N., Nweeia, M. and Ketten, D. R. Three-dimensional geometry of the narwhal (*Monodon monoceros*) flukes in relation to hydrodynamics. Society for Integrative and Comparative Biology.

2011 **Ketten**, D.R., Shoshani, J., Arruda J., Cramer S., Reidenberg J., Yamato **M.** Great ears: Functional comparisons of land and marine leviathan ears, Tetrapod Evolution: Return to Water,, San Diego, CA.

2011 Mooney, TA, S Li, D R **Ketten**, K Wang, D Wang Hearing pathways in the finless porpoise, *Neophocaena phocaenoides*, and implications for noise impacts. J. Acoust. Soc. Am. Volume 129, Issue 4, p. 2431.

2011 **Ketten**, D R, C R Williams, TA Mooney K Matassa, and K Patchet *In vivo* measures of hearing in seals via auditory evoked potentials, otoacoustic emissions, and computerized tomography. The Journal of the Acoustical Society of America 129, p. 2431.

2011 **Ketten**, D.R. Hearing Risks in Underwater Ears: What the Incidence of Hearing Impairment in Wild Animals May Tell Us about Potential Impacts. NATO Conference on Effects of Sound on Marine Mammals, Amsterdam, Netherlands.

2011 **Ketten**, D.R. Noise Impacts in Air-Adapted Ears: Mechanisms of Damage, Recovery, and Permanent Loss. International Bioacoustics Congress, La Rochelle, France

While some species are amenable to direct observation and experimentation, most are too large, too elusive, and too endangered for conventional physiological techniques.

In order to properly address these questions, we need 1) to understand the mechanisms behind strandings acknowledged to be related to man-made sounds, 2) to understand the hearing characteristics and tissue responses of the species currently known to have sustained impacts, 3) to determine the same characteristics for species in areas likely to be exposed to noise, 4) to develop robust, interactive models for responses to multiple sources and their variations in output, and 5) to disseminate these results to both the broader scientific and public arenas. To accomplish these fundamental goals, it is first necessary to find novel approaches that open a window into the physiology and anatomy of a far wider range of animals than we currently can test directly.

This proposal outlines three coordinated approaches to assist with the development and dissemination of a comprehensive, web-accessible database on the anatomy and physiology of a substantial range of marine mammals, their prey species, and other currently endangered marine species for which there are concerns for underwater acoustic impacts. The proposal requests funding for the first year of a projected multi-year effort to initiate this data base and to publish related manuals on the imaging procedures used and interpretations of the data sets.

Available material includes over 1100 image sets from 170 species ranging from invertebrates through representative marine and land mammal taxa, including approximately 400 data sets for cetaceans alone. This data base is critical to several research fronts relevant to solving acoustics impact issues, including finite element models (FEM) of whole body and auditory system impacts, the differences amongst species for common sound source responses, and the education of stranding responders and pathologists in the interpretation of images of normal variants vs pathologic states in the common range of post-mortem grades encountered in marine mammals